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RESIDUAL INFECTIOUS PANCREATIC NECROSIS (IPN) TRANSMISSION RISK FROM ARCTIC CHAR TRANSFERS INTO BRITISH COLUMBIA

1.0 Context

Infectious pancreatic necrosis (IPN) is a disease affecting some freshwater and saltwater finfish. It is caused by the infectious pancreatic necrosis virus (IPNv), which belongs to the family Birnaviridae. The IPNv can be spread by moving infected live or dead finfish, contaminated equipment, or contaminated water. Infectious pancreatic necrosis can cause death in fry, fingerlings, and smolts; including smolts transferred to seawater. There are no treatment options currently available in Canada for IPN. A number of species of fish are susceptible to, or can carry IPN including but not exclusive to:

- Cutthroat Trout (Oncorhynchus clarkii)
- Chum Salmon (Oncorhynchus keta)
- Rainbow Trout (Oncorhynchus mykiss)
- Atlantic Salmon (Salmo salar)
- Arctic Char (Salvelinus alpinus)

Because of the seriousness of the disease, it is a legal requirement to report known or suspected IPN infections to the Canadian Food Inspection Agency (CFIA). Within Canada, British Columbia is designated free of IPN, New Brunswick (NB) and Nova Scotia (NS) are considered endemic, and the virus has also been detected in many other regions of Canada.

Arctic Char (*Salvelinus alpinus*) are not indigenous to British Columbia; however, they are commercially cultured in the province on a small scale with most of the stock originating from the Yukon. While IPNv has been detected in the Arctic watershed portion of the Yukon, eggs come from an area that is considered free from the virus, and the province of BC continues to be IPN free. An application was submitted to the British Columbia Introductions and Transfers Committee in 2016 to import Arctic Char eggs from a facility in New Brunswick (an IPN endemic province), to a land based fresh water facility located in the interior of BC. Because the application indicated that waste water from the facility was direct discharge, and may enter fish bearing waters, information on the potential risk of IPNv entering aquatic environments in BC through fish introductions was needed. The facility in NB identified as the source for Arctic Char eggs held a valid fish health certificate under the DFO Fish Health Protection Regulations (FHPR) at the time this transfer was requested. Although IPNv had not been detected at the donor facility in over 10 years of testing under the FHPR, uncertainty remained regarding whether the level of testing associated with this certification would be sufficient to ensure an infection free status. Arctic Char has been shown to be a carrier of the IPN virus, often without showing symptoms of disease. Detection of IPNv in Arctic Char that are not exhibiting symptoms of the virus requires a high test sensitivity; conventional diagnostic methods will often not detect infection in sub-clinical animals. DFO Aquaculture Management Branch requested

Science Branch provide advice regarding the potential risk associated with the introduction of Arctic Char into a BC facility.

The assessment and advice arising from this Canadian Science Advisory Secretariat (CSAS) Science Response (SR), will be used to evaluate risks to wild and farmed BC fish as part of the review process for both aquaculture licence applications and Introductions and Transfers licence applications, as well as to inform decisions related to Arctic Char aquaculture conditions of licence. This Science Response Report results from the January 2017 Science Response Process on the Residual Infections Pancreatic Necrosis (IPN) transmission from Arctic Char transfers into British Columbia.

The specific objectives of this Science Response are to:

1. Document evidence of impacts in other regions where IPN has been introduced.
2. Document the likelihood of Arctic Char being infected with IPNv, and describe potential impact from an import if the Arctic Char were infected to BC wild and farmed fin fish.
3. Determine whether the risk of introducing IPNV to BC differs based on the life stage of fish being imported (e.g. eggs, milt, fry, juvenile, adult, brood stock etc.)
4. Determine the effectiveness of IPNv diagnostics and management practices in assessing virus infection status of facilities within IPNv positive areas.
5. Recommend management measures to mitigate the potential risks associated with transferring Arctic Char from facilities in IPNv positive areas
6. Identify any knowledge gaps or uncertainties associated with the advice.

This Science Response Report results from the Science Response Process April 2017 on the Residual Infectious Pancreatic Necrosis (IPN) Transmission Risk due to Arctic Char Transfers into British Columbia.

2.0 Background

2.1 General Description of the virus and its genetic types

Infectious pancreatic necrosis virus (IPNv) is the type species of the genus Aquabirnavirus (van Regenmortel et al. 2000, Delmas et al 2005) which is in the family Birnaviridae. The phylogeny of Aquabirnaviruses is shown in Figure 1. The genus is comprised of seven genogroups I to VII and two serogroups A and B (Okamoto et al 1983, Lipipun et al 1989, Hill & Way 1995, Blake et al. 2001; Cutrin et al. 2004; Nishizawa et al. 2005). Viruses within the genus are called IPNv if they are capable of causing disease in salmonid fish. Other recognized species of the genus are Tellina virus and Yellowtail ascites virus (King et al 2011, ICTV 2017). Unclassified Aquabirnaviruses are referred to as aquatic birnaviruses.

The first reported occurrence of infectious pancreatic necrosis disease was in brook trout in eastern Canada (McGonigle 1941). The viral etiology of IPN was established almost 20 years later by Wolf et al (1960). Outbreaks of the virus have been reported worldwide except for a few places such as Australia (Davies et al 2010) where IPNv is still considered to be an exotic pathogen. The World Organization for Animal Health (OIE) delisted the disease in 2005 due to its widespread distribution. In Canada, IPN is a federally reportable aquatic animal disease (CFIA 2016).

IPNv is considered endemic everywhere in Canada except British Columbia. Three IPNv genogroups are known to occur in Canada: Genogroups I, IV and V. IPNv belonging to all three genogroups has been isolated from cultured salmon and trout in New Brunswick, Nova Scotia,

Prince Edward Island and Newfoundland (McGonigle 1941, Cone & Moore 1981, Macdonald et al 1983, Kelly & Nielsen 1993, Tarrab et al 1996). IPNv belonging to Genogroup V has been isolated from hatchery-reared trout in Manitoba and wild Dolly Varden in the Yukon and Northwest Territories (Souter et al 1984, 1986). IPNv from Genogroup I has been found in wild trout in Alberta (Yamamoto 1974, Larson 1985). An aquatic birnavirus belonging to Genogroup III or a new undesignated genogroup was found in farmed salmon in British Columbia (Kieser et al 1989; Figure 1), but has not been detected since that finding.

3.0 Analysis

3.1 Impacts in other regions where IPN has been introduced - Example of the emergence of IPNv in Scotland

In Scotland, IPNv was first described in farmed rainbow trout (Ball et al 1971) and then wild and escaped farm fish (Munro et al 1976) in Loch Awe. In 1992, IPN outbreaks were reported for the first time on marine Atlantic Salmon farm sites in groups of fish 6 to 8 weeks after their transfer to seawater (Smail et al 1992). Prior to this, IPN disease had only been reported in farmed salmon during their freshwater phase. Between 1996 and 2003, IPNv prevalence increased from 30 to >80% in saltwater and from 5 to 33% in freshwater farm sites (Murray 2005, 2006a). The incidence of clinical outbreaks of IPN also increased from one case in 1996 to 39 cases in 2002 (Bruno 2004). The virus was considered ubiquitous in sea water by 2002 in most areas of Scotland (Murray et al 2003, Bruno 2004). A 2001 survey revealed an average loss of 20 to 30% of farmed salmon post-smolts which was equivalent to $3.25M annually (Canadian dollar; Anonymous 2003). In 2005, IPN disease was deregulated which eliminated official movement restrictions and compulsory slaughter policies that had previously been in place as statutory control measures.

Outbreaks of IPN in farmed Atlantic Salmon may cause an increase in the prevalence of the virus in wild marine fish (Wallace et al 2008). An investigation was conducted regarding the prevalence and persistence of IPNv in Scottish wild marine fish caught in the vicinity of aquaculture sites reporting high levels of mortality due to clinical IPN. The virus was more commonly found in benthic fish having direct contact with sediment or ingesting infected food (Wallace et al 2008). Prevalence of the virus in wild fish decreased after removal of infected fish from farm sites and fallowing the sites (Munro et al 1976, Wallace et al 2008, Murray 2006b). Wild fish reservoirs of IPNv might exist, but are insufficient to maintain prevalence in the absence of a farm (Munro et al 1976), and are not the source of outbreaks on marine and freshwater farm sites. The long term consequences of IPNv exposure on the wild fish populations are unknown.

The risk of vertical transmission and false negative diagnostic test results was considered high in Scotland. IPNv can be transmitted through contaminated eggs or milt (Wolf et al 1963). The risk of vertical transmission is higher when virus titers reach levels above the median tissue culture infectious dose (TCID50) of 104 per ml (Wolf et al 1963). Scottish scientists found only a weak correlation between virus titers in kidney and ovarian fluid but fish with high virus titers in their kidney tissue were more likely to also have high titers of virus in their ovarian fluid (Anonymous 2003). Therefore, the risk of false negative diagnostic test results was considered high since less than 1% of the broodstock fish had high virus titers. Recommended risk reduction measures for vertical transmission of IPNv in Scotland included testing 30 broodstock per site to determine if a farm was infected, testing 100% of the broodstock used and disposing of all potentially infected eggs stocks, and performing egg disinfection immediately following fertilization and again at the time of pre-hatch (Anonymous 2003).

Epidemiological models suggested that improved control of IPN disease and IPNv transmission in farmed Atlantic Salmon during the freshwater phase of their lifecycle was the most effective method for reducing the impact of IPN disease throughout the production cycle in Scotland (Murray 2005, 2006a). Using a susceptible-infected epidemic model Murray (2006a) found that on Scottish Atlantic salmon farms, the rate of infection (R0) for IPNv was 1.41 or 1.58 in freshwater and 1.45 or 1.8 in seawater sites (meaning that every infected individual was capable of infecting more than one other individual in the population). Studies showed that IPNv did not persist in marine reservoirs if farm sites were fallowed and that IPNv was short-lived in both freshwater and marine sites (Murray 2005, 2006a). Widespread distribution of the virus in Scotland was attributed to populating marine sites with infected fish from multiple freshwater sources. The high prevalence of IPNv on marine sites was attributed to infection of fish with IPNv during their freshwater phase, prior to seawater transfer (Murray 2006a). Stocking a marine site with fish originating from multiple hatcheries increases the risk of IPNv infection on that site (Murray 2006a).

3.2 Likelihood of Arctic Char being infected with IPNv, and potential impact from an import if the Arctic Char were infected to BC wild and farmed fin fish

3.2.1 Host Range and Susceptibility

IPNv and aquatic birnaviruses have a broad host range which includes salmon, trout, char and whitefish. Aquatic birnaviruses have been isolated from fish belonging to at least 32 different families, 11 species of molluscs and 4 species of crustaceans (Munro & Duncan 1977, Dorson 1982, McAllister 1983, Hill 1982, Wolf 1972, 1988, Hill & Way 1995, Reno 1999, Anonymous 2005). Many of these organisms can serve as reservoirs and vectors of IPNv; transmitting the virus within and between freshwater and marine environments.

3.2.2 Salmonids

Salmonid fish species in British Columbia known to be susceptible to IPNv are shown in Table 1. The virus has been reported in Pacific salmon in Oregon, Washington and Idaho (Parisot et al 1963, 1965, McMichael 1974, Olson et al 1994); however, IPNv has not been detected in Washington State since first being detected in Steelhead Trout in 1997. The IPNv isolates from this region belong to Genogroup I (McMichael et al 1975, Reno 1999, Blake et al 2001).

IPNv is virulent (70-100% mortality) to juvenile trout, char and salmon in freshwater (Wolf 1988) and salmon smolts during their first month after transfer to seawater (Smail et al 1992, Jarp et al 1994). Salmonid fish differ in their sensitivity to IPNv with all being highly susceptible but with brook trout being more sensitive than Rainbow Trout, Arctic Char and Atlantic Salmon (Sadasiv 1995, Dorson et al 1991). Yamamoto (1975 a, b) suggested that the higher susceptibility of Brook Trout is reflected in the higher prevalence rate and longer periods of virus persistence observed in Brook Trout populations. Lake Trout are considered to be more resistant to IPNv than many other salmonid fish species (Silim 1982, Dorson et al 1991).

Arctic Char are susceptible to IPN disease. The first report of IPNv in Arctic Char was from Sweden where apparently healthy, cultured char fry underwent disease outbreaks (Ljungberg & Jorgensen 1972). Wild Arctic Char in Germany were seropositive for IPNv-specific antibodies (Ahne et al 1989a). In Canada, IPNv has been detected in wild populations of asymptomatic anadromous Arctic Char in the Yukon and Northwest Territories west of the Mackenzie River (Souter et al 1984, 1986, Yamamoto 1989). Populations of Arctic Char in this region have since been reclassified as the Northern form of Dolly Varden (*Salvelinus malma malma*) (Kowalchuk et al 2010a&b). The virus is endemic in Dolly Varden found in tributaries of the lower Mackenzie River. IPNv isolates classified as Genogroup V have persisted in these populations

for at least 30 years (S. Clouthier, DFO, Pers. Comm.). IPNv has also been detected in Arctic Char cultured in the eastern provinces of Canada including New Brunswick (CFIA 2016).

In an experimental setting, Arctic Char fry became infected with IPNv when eyed eggs were contaminated with the virus 21 days after their fertilization (Ahne et al 1989). The eggshells remained infectious for 23 days after exposure to the virus. Arctic Char fry can be experimentally infected with IPNv 46-95 days after hatching. The mortality was less than 20% after challenge but the prevalence of the virus in survivors was as high as 82% (McAllister et al 2000). The virus titer ranged from 102.4 to 106.7 pfu/g (plaque forming units/gram) tissue. The susceptibility of Arctic char fry exposed by immersion challenge to different IPNv isolates varied with respect to cumulative mortality and virus persistence (Dorson et al 1991).

3.2.3 Non-salmonids & others

IPNv and aquatic birnaviruses are endemic in wild populations of marine and freshwater fish found around the world (Munro et al 1976, McAllister & Bebak 1997, Mortensen et al 1993, Diamant et al 1988, Wallace et al 2005). Some of those that support virus replication are shown in Table 2. A longer list of organisms in which IPNv has been detected is presented in Hill & Way (1995) and Reno (1999). Many of these organisms are present in the environment around marine and freshwater aquaculture sites in British Columbia (Kent et al 1998). Examples include shellfish (i.e. oysters, mussels, Tellina), plankton (i.e. rotifers, ciliates) and crustaceans (i.e. crab, crayfish), which may be reservoirs or vectors for the virus (Cusack & Cone 1986, Mortensen et al 1990, Hill & Way 1995, Reno 1999, Cutrin et al 2000). IPNv can persist in scallops, prawns (Mortensen et al 1992, Mortensen 1993), mussels (Molloy et al 2013), oysters (Hill 1982) and crayfish (Halder & Ahne 1988) for up to 1 year after exposure but it is not clear whether the virus is infectious. Rotifers harbour IPNv and pose a risk to the culture of halibut which are fed live rotifers during the pre-weaning phase (Comps et al 1991a,b). Parasitic ciliates carrying IPNv can transmit disease to captive populations of seahorses *Hippocampus erectus* Perry (Moewus-Kobb 1965, Cusack & Cone 1986).

Intra-species host transmission of IPNv to salmonid fish can occur. Rainbow Trout eggs and fry held in effluent downstream of IPNv-infected crayfish became infected with the virus (Halder & Ahne 1988). Mussels exposed to IPNv by bath challenge transmitted the virus to cohabiting Atlantic Salmon smolts (Molloy et al 2013). Serogroup B birnaviruses are pathogenic for rainbow trout (Hill 1982) and brook trout fry (Ahne et al 1989b), inducing typical signs of IPN disease. This virus serogroup is endemic in multiple species of flatfish and cod in the North Sea (Skall et al 2000).

3.3 Risk of infection associated with importing different life stages of fish - TRANSMISSION OF IPNv

3.3.1 Vertical transmission

IPNv can be transmitted intra-ovum or extra-ovum and can occur during the process of stripping broodstock and fertilizing ova (Anonymous 2003). Intra-ovum transmission has been demonstrated to occur in Rainbow Trout (Dorson and Torchy 1985), Brook Trout (Bullock et al 1976, Bootland et al 1991) and Arctic Char (Ahne and Negele 1985, Ahne et al 1989). IPNv can enter and survive in Atlantic Salmon eggs but evidence showing vertical transmission was inconclusive (Anonymous 2003, Smail & Munro 1989).

As mentioned in host range and susceptibility, eggshells from Arctic Char experimentally exposed to IPNv remained infected for more than three weeks (Ahne et al 1989, Ahne & Negle 1985). Attachment of the virus may occur inside or on the surface of eggs (Fijan & Giorgetti 1978, Ahne and Negele 1985, Ahne et al 1989) or on the surface of sperm cells which act as a

vehicle for virus entry into the egg via the micropyle at the time of fertilization (Mulcahy & Pascho 1984). Both methods have been suggested for transmission of IPNv in salmonid fish. The virus has been isolated from milt (Ahne 1983, Bootland et al 1991, Smail & Munro 2008) and ovarian fluid (Wolf et al 1963, Bootland et al 1991, Smail & Munro 2008) originating from asymptomatic salmonid fish.

Disinfection of fertilized eggs with iodophores reduces but does not eliminate vertical transmission of the virus (Ahne et al 1989, Bullock et al 1976, Dorson et al 1997). IPNv shows a strong affinity to the eggshell and upon attachment may be protected from disinfectants by the physical changes in the eggshell that occur during the hardening process (Ahne et al 1989, Bullock et al 1976). IPNv can survive up to 4 months in the perivitelline space of dry-fertilized eggs from Atlantic Salmon, posing a significant risk of vertical transmission in this species (Smail & Munro 1989). The virus also absorbs quickly and easily to the surface of spermatozoa (Dorson & Torchy 1985, Smail & Munro 1989, 2008, Ahne et al 1989). According to Ahne et al (1989), the threat of vertically transmitting IPNv can be lowered by disinfecting milt because virus particles on spermatozoa are accessible to disinfecting agents. Egg disinfection immediately post-fertilization and again pre-hatch may reduce the risk and prevent transmission (Anonymous 2003).

The risk of vertical transmission rises as the quantity of virus increases in reproductive products (Dorson & Torchy 1985, Bootland et al 1991, 1995). IPNv titers are higher in the ovarian fluid than egg homogenates (Wolf et al 1963.) which is the rationale for diagnostic testing of ovarian fluid for detection of IPNv. The threshold titer for vertical transmission of IPNv in ovarian fluid from brook trout was 104 TCID50 per ml (Wolf et al 1963). Atlantic salmon ovarian fluid may contain IPNv neutralizing factors such as vitellogenin or anti-IPNv antibodies which may increase the virus titer required for successful vertical transmission (Gregory et al 2003, Garcia et al 2010).

3.3.2 Horizontal transmission

3.3.2.1 Horizontal transmission factors

3.3.2.1.1 Minimum infectious dose

IPNv can be transmitted to salmonid fish by exposing them to a suspension of IPNv in water, cohabitating them with virus-infected fish, or injecting them (intra-peritoneal [ip]) with virus (McAllister & Owens 1986, Bootland et al 1991, Bowden et al 2002, Munang'andu et al 2016). In the cohabitation challenge model, naïve fish are exposed to virus shed in the faeces and urine of the infected fish (Billi & Wolf 1969, Frantsi & Savan 1971b). IPNv infection dynamics are influenced by host species, genetic background, life stage, challenge method, virus isolate and dose. The minimum dose required for infection and mortality in Atlantic salmon smolts was 10^{-1} TCID50 per ml after bath exposure (Urquhart et al 2008) or 10 TCID50 per fish after injection challenge in seawater (Bowden et al 2002). In the first study, virus shedding from acutely infected fish occurred over 12 days peaking on day 11 at an estimated rate of 6.8×10^{3} TCID50 per hour per kg (Urquhart et al 2008). The experiment suggested that the peak rate of virus shedding coincided with the first observed mortalities. Fecal shedding rates in brook trout exposed to IPNv by ip injection can reach approximately 10^{5} TCID50 per fish and virus can still be detected in feces 76 weeks after infection (Bootland et al 1991).

3.3.2.1.2 Virus Stability

IPNv is among the most chemically and environmentally stable fish viruses and its stability contributes to the high risk associated with horizontal transmission of the virus (Myrmel et al 2014). The efficacy of various treatments for inactivating IPNv is presented in Table 4. IPNv is insensitive to treatment with chloroform or ethyl ether (Malsberger & Cerini 1963, Parisot et al

1965) and exposure to pH 3.0 for 1 hour or heating at 60°C for 30 minutes (Ahne 1982, Reno 1999, Nygaard et al 2012). The virus is pH tolerant (Smail et al 1993a, Whipple & Rohovec 1994, Dixon et al 2012b) but it can be inactivated by base treatment above pH 11.5 or by acid treatment below pH 1.5 (Table 4; Ahne 1982, Myrmel et al 2014, Dixon et al 2012a).

3.3.2.2 Reservoirs and vectors for horizontal transmission

3.3.2.2.1 Wild aquatic animals

IPNv has been isolated from a number of freshwater and marine animal species which are considered to be reservoirs of the virus (see section on host susceptibility for further discussion). Table 3 presents a list of species that are known to carry the IPNv in either reproductive fluids or other tissues.

3.3.2.2.2 Convalescent fish

Adult fish that survive infection can serve as lifelong asymptomatic carriers of the virus (Reno et al 1978, Swanson & Gillespie 1979, Smail & Munro 1985) shedding virus particles in their urine, faeces and reproductive products (Yamamoto 1975a, b, Ahne 1983, Bootland et al 1995). The IPNv titer in these fish has been shown to fluctuate with time from non-detectable to relatively high (Billi and Wolf 1969, Yamamoto 1975a, b, Hedrick and Fryer 1982, Mangunwiryo and Agius 1988, Bootland et al 1991). Virus transmission from these fish in the wild is considered to be infrequent given the low prevalence of IPNv found in cohabiting trout and wild progeny after the addition of adult carrier brook trout to a natural isolated lake in Alberta, Canada (Yamamoto & Kilistoff 1979).

Recurrence of IPN in covertly infected fish can occur under experimental conditions by exposure to environmental stress (rainbow trout & Atlantic salmon; Roberts & McKnight 1976, Taksdal et al 1998). Recrudescence from persistent infections to active infections in Atlantic Salmon has also been observed under field conditions (Atlantic salmon: Smail et al 1992, Mutoloki et al 2016) and has been attributed in part to the physiological stress of smoltification and the stress of transport from the hatchery to seawater netpens (Jarp et al 1994, Murray et al 2003). In these cases, the probability of IPN outbreaks and the severity of mortality in the seapens were higher when cohorts came from hatcheries where IPN had been detected (Jensen & Kristoffersen 2015).

The site of persistent infection for IPNv in asymptomatic carriers is unknown but the virus has been found in peripheral blood leucocytes of trout (Rainbow Trout, Brook Trout: Agius et al 1982, Yu et al 1982, Swanson & Gillespie 1982, Ahne & Thomsen 1986, Mangunwiryo & Agius 1988, Ahne et al 1989a, Saint-Jean et al 1991), Atlantic Salmon (Knott & Munro 1986, Johansen & Sommer 1995, Munro et al 2004, 2006) and Atlantic cod (Martin-Armas et al 2007). Virus titers in these fish tend to be higher in the kidney tissue which contains the majority of leuco- and haemopoietic cells of the host (Munro & Duncan 1977, Yamamoto 1974, 1975a).

3.3.2.2.3 Water

Virus dispersion through water movement can play a significant role in the transmission of IPNv especially given that the virus is very stable in the aquatic environment. IPNv is more stable in saline water but can survive for extended periods of time in untreated freshwater, estuarine water and sea water (Toranzo & Hetrick 1982, Desautels & MacKelvie 1975). The length of time required to reduce the titer of IPNv by 99.9% was 17 days in freshwater, 27 days in estuarine water and 17 days in sea water at 15°C (Toranzo & Hetrick 1982). Filtration or autoclaving of water samples reduced the rate of virus inactivation suggesting that resident microbial flora may have an antiviral effect (Toranzo & Hetrick 1982, Toranzo et al 1983). Lower water temperatures increased survival times of the virus (Toranzo & Hetrick 1982). This

may explain the results from an earlier study in which IPNv required 10 to 12 weeks before it lost 99% of its infectivity in fresh water at 4°C. Residual infectivity was detected up to 24 weeks after the epizootic (Desautels & MacKelvie 1975). In seawater, the virus persisted with negligible titer reduction after 10 weeks and less than 99% reduction after 5 to 6 months at 4°C and 10°C (Desautels & MacKelvie 1975). In both cases, IPNv exhibited greater stability in saline water which may play a contributory role in the pathogenicity of aquatic birnaviruses for estuarine organisms (Toranzo & Hetrick 1982).

3.3.2.2.4 Farmed fish & fish farming activities

Virus-infected live fish in a hatchery or marine netpen site are potential sources of virus that can be shed into the surrounding environment. The persistence of IPNv in freshwater and sea water increases the spatial and temporal hazard risk to other fish in the area. Resident wild fish located 19.3 km downstream were infected with IPNv (10^{-1} pfu/ml) from a hatchery releasing effluent during an epizootic outbreak of disease in Rainbow Trout (10 pfu/ml) (McAllister & Bebak 1997). A similar situation occurred in Scotland except that the virus levels in the hatchery effluent were as high as 10^{4} pfu/ml (Munro et al 1976). Resident wild fish tested positive for the virus suggesting that waterborne transmission occurred via effluent from the hatchery. There is also evidence of horizontal transmission from clinical outbreaks of IPN disease in farmed Atlantic Salmon on marine sites in Scotland. A localized increase in the prevalence of IPNv was observed in wild marine fish located within 5 km of IPN-positive farms (i.e. 0.15% versus 0.58%) (Wallace et al 2008). A higher proportion of wild benthic fish tested positive which may be related to their preference for substrates present below and around aquaculture sites in combination with the 100-fold accumulation of IPNv reported to occur in sediment versus water in the vicinity of sites experiencing epizootics (Gregory et al 2007). Epidemiological modelling suggests that the most significant risk factor for outbreaks of IPN on Atlantic Salmon marine farms in Norway was the cohort size put to sea (Jensen & Kristoffersen 2015). The probability of IPN outbreaks and the severity of mortality in the seapens were higher when cohorts came from hatcheries where IPN had been detected. The number of fish stocked was predicted to increase the infection pressure and raise the risk associated with horizontal transmission of the virus to other pens on site (Jensen & Kristoffersen 2015).

Piscivorous birds and mammals can also function as vectors of IPNv after feeding on live or dead fish. Faecal samples collected from wild herons and mallards at three salmonid fish hatcheries contained IPNv at titers equivalent to those found in tissues from fish reared in these hatcheries (McAllister & Owens 1992). IPNv was also found in excrement but not spleen tissue from a chicken and great horned owl intubated with IPNv and from mink feeding on IPNv-infected tissue (Sonstegard & McDermott 1972). Cows fed a composite fish and grass silage inoculated with IPNv shed the virus in their faeces for 3 days following the start of feeding (Smail et al 1993b). These studies suggest that IPNv can survive enteric passage in warm blooded animals. As such, they may be a mechanical vector capable of passively transferring the virus to farmed fish.

IPNv can also be spread through anthropogenic activities. During fish farming, water, equipment, clothing and transport vessels that come in contact with infected fish can subsequently be a source of IPNv transmission (Murray et al 2002).

3.4 Factors That Influence Host Susceptibility

The severity of IPNv disease is influenced by a number of factors including virus isolate, host species, genetics, age, exposure status and environmental conditions (Wolf 1988, Guy et al 2006, Wetten et al 2007, Kjoglum et al 2008). Host species and life stage are discussed here.

3.4.1 Host species

Differences in host species susceptibility have been observed with Brook Trout which are more sensitive to IPN disease than Rainbow Trout, Arctic Char and Atlantic Salmon (Sadasiv 1995, Dorson et al 1991). Brook Trout are also more susceptible than Chinook Salmon, Sockeye Salmon, kokanee and Coho Salmon (Parisot et al 1963). Lake Trout are the least susceptible to IPNv of these salmon species (Silim 1982, Dorson et al 1991).

3.4.2 Host lifestage

The age and immunological development of a host influences its susceptibility to IPN disease. Clinical symptoms and mortalities appear in fry 2 to 4 weeks after their first feeding. Fish older than 5 to 6 months are susceptible to infection but not disease (Brook Trout, Rainbow Trout, Atlantic Salmon; Wolf et al 1960, Elazhary et al 1976, Ahne et al 1989, Frantsi & Savan 1971a, Swanson & Gillespie 1979, Dorson & Torchy 1981, LaPierre et al 1986, McAllister & Owen 1986). A carrier state can be established in older fish (Wolf & Quimby 1969, Reno et al 1978, Swanson & Gillespie 1979). Some salmonid fish, particularly Brook Trout, can become lifetime carriers of the virus (Billi & Wolf 1969, Yamamoto 1975b, Yamamoto & Kilistoff 1979). IPNv has been isolated from Arctic Char reproductive products, fry and adults during their freshwater stage. Evidence for the presence of IPNv in the reproductive fluids and tissues from fry, smolts and adult lifestages of salmonid fish species found in British Columbia is shown in Table 3.

The high thermal tolerance explains why IPNv can survive enteric passage in birds, mink, and cows feeding on IPNv-infected material, thereby creating unexpected mechanical vectors for this virus (Sonstegard & McDermott 1972, Jorgensen 1973, McAllister & Owens 1992, Smail et al 1993b). The virus can be recovered after 1 year at 4°C or 2 months at 15°C in buffered solution (Dorson 1982). IPNv can survive dessication in a controlled laboratory setting with residual activity detected after 8 weeks. Humidity increased the rate of inactivation such that no infectivity was detected after 5 weeks (Desautels & MacKelvie 1975). In a hatchery setting, IPNv remained infective after air-drying for 2 but not 3 weeks at 15 to 22°C (Wolf 1966).

3.5 Effectiveness of Current Testing for IPNv

IPN is a federally reportable disease in Canada. It is listed under the country's Health of Animals Act and Regulations which govern the National Aquatic Animal Health Program (NAAHP). The NAAHP is co- delivered by the Canadian Food Inspection Agency (CFIA) and Fisheries and Oceans Canada (DFO). CFIA is the lead regulatory authority responsible for conducting surveillance and responding to reports of disease outbreaks for the purposes of domestic disease control and international trade. Diagnostic testing performed on behalf of Canada's NAAHP is carried out by three member laboratories of NAAHLS run by Fisheries and Oceans Canada (DFO). The national reference laboratory for IPNv is located at the Freshwater Institute (Winnipeg, MB). Testing for this pathogen is also conducted at the Pacific Biological Station (Nanaimo, BC) and the Gulf Fisheries Center (Moncton, NB).

The National Aquatic Animal Health Laboratory System (NAAHLS) member laboratories use a combination of diagnostic tests to detect IPNv. Samples collected from apparently healthy populations are screened using a DNA amplifying Polymerase Chain Reaction (RT-qPCR) test that targets a section of the IPNv genome encoding the structural protein VP2 and amplifies a 116 base pair fragment located between nucleotides 141 and 256 of genome segment A (numbering as presented in Genbank accession number M18049.1). The test method has been shown to be sensitive (i.e. minimum limit of detection is 20 copies of the IPNv VP2 gene), specific (i.e. does not amplify nucleic acid from viral pathogens that may be co-existing with IPNv) and almost universal in that it is capable of detecting the North American isolates represented by Genogroups I, IV and V as well as representatives from two of the remaining

four genogroups (i.e. (III & VII). The target tissue is kidney although other suitable tissues or fluids can be used. Since CFIA would require RT-qPCR testing for IPNv under a test and ship scenario, this would address concerns of the BC ITC of false negative test results that could occur from asymptomatic carriers of IPNv under the traditional testing method of virus isolation prescribed by the FHPR Manual of Compliance. If virus is detected or if samples are from populations undergoing a disease outbreak, virus isolation by cell culture (VI) is included in the diagnostic pathway. The CHSE-214 cell line displays higher sensitivity to IPNv than Bluefin gill (BF-2), RTG-2, FHM and epithelioma papulosum cyprinid (EPC) cell lines (Lorenzen et al 1999, McAllister 1997, Rodriguez et al 1993). Whole fish (<4 cm), a pool of kidney, gill and spleen tissue, or reproductive fluids can be submitted for testing.

The performance of both tests has been validated to assess their fitness as diagnostic tools for detection of IPNv. Test performance metrics of diagnostic accuracy were sensitivity (DSe) and specificity (DSp). Repeatability and reproducibility were measured to assess diagnostic precision. Estimates of test accuracy, in the absence of a gold standard reference test, were generated using latent class models and gold standard reference populations. Moderate to high repeatability (79 to 89%) and reproducibility (74 to 89%) were observed for the RT-qPCR and VI tests. Precision of both tests was reduced when samples were from an apparently healthy population with a low virus load. In this case, estimates for kappa for the cell culture test were in the slight to moderate agreement categories whereas those for the RT-qPCR test were in the fair to substantial agreement categories. High accuracy estimates of ≥97% for DSe and ≥98% for DSp were obtained for both tests when samples were from naïve and diseased populations, respectively. The estimates generated for the RT-qPCR test using samples from apparently healthy populations remained high at 83 to 91% for DSe and 86 to 92% for DSp. Poor accuracy was observed for the virus isolation test with samples from the same population (i.e. 22 to 27% for DSe and 87 to 90% for DSp). The results indicated that the cell culture test is not a suitable tool for detection of IPNv in apparently healthy populations given the high probability of false negative results. The RT-qPCR test is considered to be a suitable diagnostic test for detection of IPNv in apparently healthy populations and those experiencing a disease outbreak. It is 61 to 64% more likely than the cell culture test to detect the virus in fish with a low virus load. A manuscript describing the results of the validation study is in preparation (Clouthier, Pers. Comm).

3.5.1 BC fish health surveillance for detection of IPNv

The fish health status of Atlantic and Pacific salmon reared in marine and freshwater environments throughout BC was evaluated under the Fish Health Audit and Surveillance Program run initially by the British Columbia Minister of Agriculture and subsequently by DFO. Additional fish health monitoring programs were implemented by the aquaculture industry in the province. The government program conducted 550 farm audits and performed 3,183 tests for detection of IPNv between 2006 and 2011. The program operated by the aquaculture industry completed 2,737 tests for detection of the virus during the same time period. A combination of molecular tests, virus isolation by cell culture and histopathology were used. All testing was negative for IPNv. A quantitative epidemiological evaluation of these surveillance programs by the CFIA revealed that the mean probability of disease freedom for IPNv in BC was greater than 99% and that additional surveillance activities beyond those already in place were not required to substantiate the absence of IPNv in BC farmed salmon. The latter statement was conditional on the continued practice of import and domestic movement controls to prevent the introduction of IPNv into the rearing process (CFIA 2014).

3.5.2 CFIA surveillance 2012 to 2015.

The freedom of IPN disease status of British Columbia was substantiated by CFIA through a surveillance program that was initiated in 2012 by the agency's Aquatic Surveillance and Epidemiology Section. Juvenile and adult life stages of wild and cultured Pacific salmon (n=1,189) were tested for IPNv between 2012 and 2014 (CFIA 2014) and again in 2015. The virus was not detected in any of the samples (Table 4).

3.5.3 IPNv in Atlantic Canada

IPNv is endemic in eastern Canada, a status that has been recognized for more than 75 years since IPNv was first described in the region (McGonigle et al 1941). Earlier reports of disease outbreaks with clinical signs consistent with IPN date back to 1906 (McGonigle et al 1941). The current fish health status of farmed and wild Atlantic salmon and trout in Ontario, Quebec and the maritime provinces have been assessed through diagnostic testing conducted at NAAHLS member laboratories in support of CFIA surveillance, facility certification or response to disease, planned movement of live aquatic organisms and import/export of aquatic animals. Results from testing conducted between 2012 and 2016 indicate that IPNv persists in the regional freshwater and marine aquatic environments. IPNv has been detected in Arctic Char from New Brunswick annually since at least 2005. A summary of the results is presented in Table 5.

3.6 Recommended management measures to mitigate the potential risks associated with transferring Arctic Char from facilities in IPNv positive areas

Steps suggested for reducing the prevalence of IPNv in farmed Atlantic salmon during their freshwater phase include maintaining high biosecurity at freshwater facilities, screening populations of fish in freshwater prior to saltwater transfer and minimizing transportation distances where practical (Wallace et al 2016). Results from modeling studies suggest an effective IPNv management strategy would incorporate an area-based rather than a site-level approach in combination with husbandry measures taken to limit clinical disease in infected sites (Murray 2005, 2006a).

The disposal of dead fish collected during a farm production cycle represents another potential source of disease transmission. An alkaline hydrolysis method in which macerated fish mortalities are exposed to high pH (>13) for 7 days inactivates high titers of IPNv and is recommended as a biosecure treatment method for fish by-products that contain fish pathogens (Table 4; Dixon et al 2012a). However, ensiling (a method of carcass disposal that involves lowering the pH to <4) was determined to not be a biosecure method for disposal of fish mortalities (Table5; Smail et al 1993a, Dixon et al 2012b). Addition of Virkon to IPNv-infected silage inactivates the virus but renders the material unsuitable as an animal feedstuff (Smail et al 1993a).

Disinfection is the most effective risk reduction measure that can be taken against IPNv (Amend 1976). Iodophors and chlorine are among the most common disinfectants used in the aquaculture industry. They display virucidal activity against IPNv but their efficacy is dependent on the virus dose, contact time, presence of organic matter and water pH (Table 5; Desautels & MacKelvie 1975, Elliott & Amend 1978). The recommended dose for surface disinfection of clean surfaces in a hatchery including nets, boots and tools is 50 to 200 mg/liter for 5 to 30 minutes (Meyers 2014, Wedemeyer 2001). Another common hatchery practice is to subject infected farm equipment to a period of drying, the length of which would have to take in account the relative stability of IPNv to dessication.

It has been a long-standing policy in British Columbia that eggs are the only life stage allowed to be imported into British Columbia and these eggs must be disinfected prior to shipping and

again upon arrival into an approved quarantine facility. As is noted in Table 5, the use of an iodophor is prescribed for the disinfection of eggs prior to shipping. It is generally accepted by the industry that drying is not an effective practice for inactivating IPNv (Anonymous 2003). Chlorination of the water is the most frequently used disinfection methods for mitigating the risk of introducing virus via the transport water, maintaining it at a dose and time consistent with the known literature keeping in mind that disinfectants should be effective for the most resistant pathogen.

4.0 Conclusions

The IPN virus is considered ubiquitous throughout North America and Europe, but has not been detected in British Columbia. Maintaining this infection free status is important for international trade as well as minimizing the effects that this virus could have on native stocks of salmonids and other fish of importance to the BC economy.

The following steps are considered adequate to reduce or mitigate the potential risk associated with importing Arctic Char eggs from an IPNv endemic area to British Columbia:

1. Importing only eggs that have been surface disinfected at the source facility before transport to BC, as well as upon arrival at the receiving facility, has been shown to greatly reduce the risk that IPNv will be present in egg stock (50ppm Iodophore for 10 minutes).
2. Test source facility animals using RT qPCR methods by an accredited laboratory to ensure a correct diagnosis of the infection state of the animals is obtained, to ensure sample integrity.
3. Implement a strict Biosecurity Plan in the receiving facility to ensure effluent is not discharged into natural waters.
4. Establish escape prevention measures in the receiving facility to reduce the risk of transferring IPNv into BC waters.
5. Conduct regular health checks of the stocks that result from the import of eggs, using RT qPCR methods, to check for asymptomatic carriers.

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Appendix 1

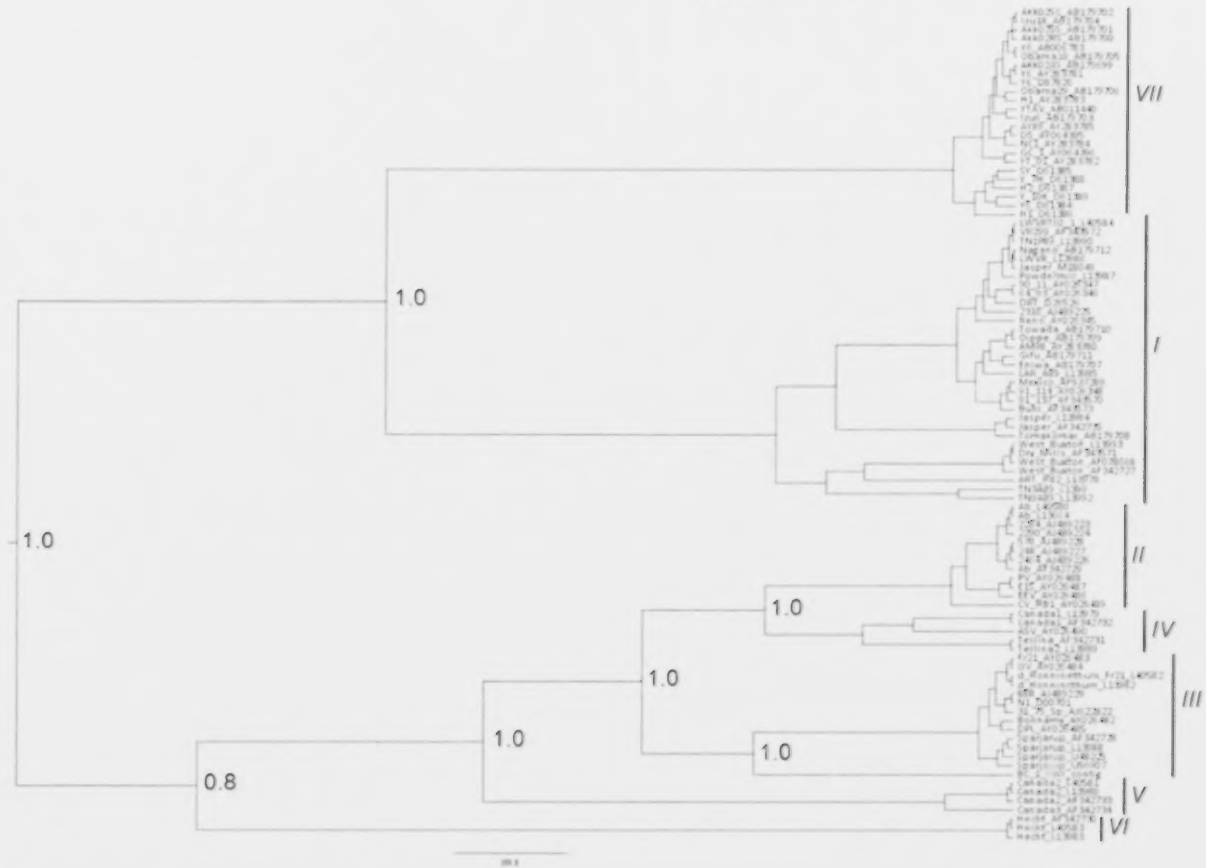

Figure 1. Phylogeny of IPNv isolates. The tree is based on the 310 bp VP2-NS junction sequence analyzed by Bayesian coalescent MCMC methods. Posterior probability values are provided at the major nodes leading to each of the seven genogroups. The tips are labelled with the isolate name and the Genbank accession number for the sequence. Genogroups are numbered I to VII (Nishizawa et al 2005).

Table 1. Salmonid species susceptible to IPNv infection (from Munro & Duncan 1977, Reno 1999, Anonymous 2005, McAllister 1983, Wolf 1972, Dorson 1982 and other literature)

Atlantic Salmon		
Common name	**Scientific name**	**Reference**
Atlantic salmon	*Salmo salar L.*	MacKelvie and Artsou 1969
Pacific salmon		
Common name	**Scientific name**	**Reference**
Chinook salmon	*O. tshawytscha*	Tisdall & Phipps 1987
Chum salmon	*O. keta*	Hah et al 1984
Coho salmon	*O. kisutch*	Wolf & Pettijohn 1970, McMichael et al 1973, 1975, Olson et al 1994
Kokanee salmon	*O. nerka*	Sano 1973
Pink salmon	*O. gorbuscha*	Dorson 1982, McAllister 1983
Sockeye salmon	*O. nerka*	Yasutake et al 1965
Char		
Common name	**Scientific name**	**Reference**
Lake trout	*Salvelinus namaycush*	Silim et al 1982
Arctic char	*S. alpinus*	Ljungberg & Jorgensen 1972, Dorson et al 1991, McAllister et al 2000
Brook trout	*S. fontinalis*	Snieszko et al 1959; Wolf et al 1960, Parisot et al 1963
Dolly varden	*S. malma*	Souter et al 1984, 1986
Japanese char	*S. pluvius*	Hill 1982
Trout		
Common name	**Scientific name**	**Reference**
Rainbow trout	*O. mykiss*	Parisot et al 1963, Fijan 1974,
Amago trout	*O. rhodurus*	Sano 1973, Sano & Yamazaki 1973
Biwa trout	*O. masuo rhodurus*	Sano 1973
Brown trout	*S. trutta*	Snieszko et al 1959, McKnight & Roberts 1976
Cutthroat trout	*O. clarkii*	Parisot et al 1963, McMichael et al 1975
Others		
Common name	**Scientific name**	**Reference**
Grayling	*Thymallus thymallus*	Ahne 1980, Dorson 1982, McAllister 1983
Huchen	*Hucho hucho*	Ahne 1980, Dorson 1982, McAllister 1983

Table 2. Non-salmonid species susceptible to IPNv infection (from McAllister 1983, Dorson 1982 and updates from the literature)

Freshwater fish

Common name	Scientific name	Reference
Barbel	*Barbus barbus*	Ahne 1980, Dorson 1982, McAllister 1983
Common bream	*Abramis brama*	Adair & Ferguson 1981
Common carp	*Cyprinus carpio*	Ahne 1980, Daud & Agius 1987
Common roach	*Rutilus rutilus*	Dorson 1982, McAllister 1983
Discus fish	*Symphysodon discus*	Adair & Ferguson 1981
Goldfish	*Carassius auratus*	Adair & Ferguson 1981
Northern pike	*Esox lucius*	Ahne 1978, 1980
Tilapia	*Oreochromis spilurus*	Agius 1982, Chen et al 1985, Mangunwiryo & Agius 1988
Walleye	*Stizostedion vitreum vitreum*	Schat & Carlisle 1980, McAllister 1983
Zebra danio	*Brachydanio rerio*	Seeley et al 1977, Dorson 1982

Marine fish

Common name	Scientific name	Reference
Atlantic menhaden	*Brevoortia tyrannus*	Stephens et al 1980
Atlantic shad	*Alosa sapidissima*	Stephens et al 1980
Spotted wolffish	*Anarhichas minor*	Sommer et al 2004
Striped bass	*Morone saxatilis*	Schutz et al 1984, Wechsler et al 1986, 1987

Marine groundfish

Common name	Scientific name	Reference
Atlantic cod	*Gadus morhua*	Skall et al 2000, Jensen et al 2009, Urquhart et al 2009
Atlantic halibut	*Hippoglossus hippoglossus*	Mortensen et al 1990, Biering et al 1994
European eel	*Anguilla anguilla*	Hudson et al 1981, McAllister 1983
Haddock	*Melanogrammus aeglefinus*	Ruane et al 2007, Anonymous 2005
Japanese eel	*Anguilla japonica*	Sano et al 1981, Lee et al 1999
Southern flounder	*Paralichthys lethostigma*	McAllister 1983
Turbot	*Scophthalmus maximus*	Castric et al 1987, Mortensen et al 1990, 1993

Table 3. Freshwater (FW) and marine (SW) fish species in British Columbia that have been documented elsewhere to have IPNv in reproductive products or tissues from different lifestages.

Species	Reproductive products	Lifestage				
		Fry	Smolts		Adult	
			FW	SW	FW	SW
Arctic char *Salvelinus alpinus*	√[1,10,11]	√[1,2,10,11, 16]	-	-	√[1]	-
Atlantic salmon *Salmo salar*	√[8,9]	√[8,13]	√[8]	√[7,12]	√[9]	√[8]
Brook trout *S. fontinalis*	√[32,33]	√[4,14,18]	-	-	√[6,20]	-
Brown trout S. trutta	-	√[31,34]	-	-	-	-
Chinook salmon *Oncorhynchus tshawytscha*	-	-	-	-	√[24,26]	-
Chum salmon *O. keta*	√[25]	-	-	-	-	-
Coho salmon *O. kisutch*	-	√[15]	-	-	√[26,30]	-
Cutthroat trout *O. clarki*	-	√[19]	-	-	√[26]	-
Dolly varden *S. malma*	√[22]	-	-	-	√[17,23]	√[17,23]
Kokanee *O. nerka*	-	√[27]	-	-	-	-
Lake trout *S. namaycush*	√[3]	√[4,14]	-	-	√[3]	-
Pink salmon *O. gorbuscha*		√[28,29*]				
Rainbow trout *O. mykiss*	√[10,11]	√[4,8,14]	-	-	√[5,8]	-
Sockeye salmon *O. nerka*	-	√[21]	-	-	-	-

1, Ahne et al 1989; 2, Ljungberg & Jorgensen 1972; 3, Larson 1985; 4, Shankar & Yamamoto 1994, 5, Yamamoto 1975a; 6, Yamamoto 1975b; 7, Stangeland et al 1996; 8, Krogsrud et al 1989; 9. Smail & Munro 1989; 10, Ahne & Negle 1985; 11, Ahne et al 1989; 12, Smail et al 1992; 13, MacKelvie & Artsou 1969; 14, Silim et al 1982; 15, Wolf & Pettijohn 1970; 16, McAllister et al 2000; 17, Yamamoto 1989; 18, McAllister & Owens 1986; 19, Parisot et al 1963; 20, Sonstegard & McDermott 1971; 21, Yasutake et al 1965; 22, Souter et al 1984; 23, Souter et al 1986; 24, Tisdall & Phipps 1987; 25, Hah et al 1984; 26, McMichael 1974; 27, Sano 1973; 28, McAllister 1983; 29, Dorson 1982; 30, Olson et al 1994; 31, Snieszko et al 1959; 32, Wolf et al 1963; 33, Wolf et al 1968; 34, McKnight & Roberts 1976.

* lifestage not specified

Table 4. IPNv RT-qPCR test results for Pacific salmon and trout evaluated through the CFIA surveillance program in British Columbia between 2012 and 2015

Common name	Scientific name	Number of fish tested					
		Hatchery			Wild		
		Juvenile	Adult	Total	Juvenile	Adult	Total
Chinook salmon	*Oncorhynchus tshawytscha*	0/404	0/514	0/918	0/296	0/573	0/869
Chum salmon	*O. keta*	0/327	0/271	0/598	0/251	0/486	0/737
Coho salmon	*O. kisutch*	0/790	0/823	0/1613	0/258	0/935	0/1193
Pink salmon	*O. gorbuscha*	0/0	0/292	0/292	0/250	0/220	0/470
Sockeye salmon	*O. nerka*	0/0	0/0	0/0	0/332	0/477	0/809
Steelhead salmon	*O. mykiss*	0/234	0/214	0/448	0/332	0/128	0/460
Cutthroat trout	*O. clarkii*	0/0	0/0	0/0	0/0	0/1	0/1
	Totals	0/1755	0/2114	0/3869	0/1719	0/2820	0/4539
	Grand total	**0/16,816**					

Table 5. IPNv test results for wild and cultured Atlantic salmon and trout sampled in eastern Canadian provinces between 2012 and 2016

Common name	Scientific name	Number of fish tested					
		Hatchery			Wild		
		Juvenile	Adult	Total	Juvenile	Adult	Total
Arctic char	*Salvelinus alpinus*	30/5030*	0/749	30/5779	-	-	-
Atlantic salmon	*Salmo salar*	1/1188	0/683	1/1871	-	-	-
Brook trout	*Salvelinus fontinalis*	-	-	-	0/167	-	0/167
Lake trout	*Salvelinus namaycush*	0/175	-	0/175	-	-	-
Rainbow trout	*Oncorhynchus mykiss*	0/770	0/773	0/1543	0/222	-	0/222
	Totals	1/2133	0/1456	1/3589	0/389	-	0/389
	Grand total			31/3978			

*Data is taken from testing under the Fish Health Protection Regulations certification program. All data is from pooled viral assays. Of the 1208 tissue pools tested, 30 pools were presumptively positive for IPNv (showing IPNv-like CPE in original VI assay). Of those 30 presumptive positive, 7 pools were confirmed by subculture and molecular testing.

Table 6. Efficacy of IPNv disinfection treatments (see also Husby 2003, Bovo et al 2005)

Method	Dose	Time	Notes	Reduction	Reference
Heat	60°C	1-2 d	Isolate dependent; 3.75% bovine serum albumin	100%	Dixon et al 2012b
	60°C	8 hr	MEM-0	100%	Whipple & Rohovec 1994
	65°C	3.5 hr	MEM-0	100%	Whipple & Rohovec 1994
	70°C	2 hr	MEM-0	100%	Whipple & Rohovec 1994
	80°C	10 min	MEM-0	100%	Whipple & Rohovec 1994
Temperature & low pH	4°C + pH 4	147 d	Tris-glycine acid buffer pH 3.8; $10^{6.48 \text{ to } 8.3}$ pfu/ml	28%	Smail et al 1993a
	4°C + pH 4	58 wks	Silage; $10^{5.91 \text{ to } 7.5}$ pfu/ml	27%	Smail et al 1993a
	20°C + pH 4	71 d	Tris-glycine acid buffer pH 3.8; $10^{6.48 \text{ to } 8.3}$ pfu/ml	99.9%	Smail et al 1993a
	20°C + pH 4	71 d	Silage; $10^{5.91 \text{ to } 7.5}$ pfu/ml	99.9%	Smail et al 1993a
	45°C + pH 4	5 hr	Silage; $10^{5.91 \text{ to } 7.5}$ pfu/ml	24 to 47%	Smail et al 1993a
	60°C + pH 4	1 hr	Silage; $10^{5.06}$ pfu/ml	51%	Smail et al 1993a
	60°C + pH 4	1 hr	Silage; $10^{2.78}$ pfu/ml	100%	Smail et al 1993a
	65°C + pH 4 (30 to 180 min tested)	180 min		36%	Whipple & Rohovec 1994
	65°C + silage pH 4 (30 to 180 min tested)	70 min		100%	Whipple & Rohovec 1994
	65°C, 15 min then 82°C, 5, 10 min + pH 4	5 min		100%	Whipple & Rohovec 1994
	65°C, 15 min then 82°C, 5, 10 min + silage pH 4	5 min		100%	Whipple & Rohovec 1994
Iodophor	16 ppm (2 to 32 ppm tested)	5 min	Wescodyne; $10^{6.3}$ $TCID_{50}$/ml	100%	Amend & Pietsch 1972
	30 ppm (10 to 45 ppm tested)	5 min	Wescodyne; $10^{5.5}$ $TCID_{50}$/ml	100%	Desautels & MacKelvie 1975
	35 ppm (10 to 45 ppm tested)	5 min	Wescodyne; $10^{5.6}$ $TCID_{50}$/ml	100%	Desautels & MacKelvie 1975
	32 ppm (2 to 32 ppm tested)	5 min	Betadine; $10^{6.3}$ $TCID_{50}$/ml	100%	Amend & Pietsch 1972
	4 mg per liter (0.5 to 64 mg/l tested)	5 min	Betadine ; distilled water; $10^{3.9}$ $TCID_{50}$/ml	100%	Elliot & Amend 1978
	16 mg per liter (0.5 to 64 mg/l tested)	5 min	Betadine ; distilled water; $10^{6.3}$ $TCID_{50}$/ml (pH 6-8.6; 0-300 mg/l $CaCO_3$; 0.5-8% calf serum)	100%	Elliot & Amend 1978
Chlorine	1 mg per liter (0.13-16 mg/l tested)	5 min	distilled water; $10^{3.9}$ $TCID_{50}$/ml	100%	Elliot & Amend 1978
	4 mg per liter (0.13-16 mg/l tested; pH 6.6-8)	5 min	distilled water; $10^{4.5}$ $TCID_{50}$/ml	100%	Elliot & Amend 1978
	16 mg per liter (0.25-16 mg/l tested; pH 9-10)	5 min	distilled water; $10^{4.5}$ $TCID_{50}$/ml	100%	Elliot & Amend 1978
	19-25 mg per liter (0-50 mg/l tested; 0-4% calf sera)	30 min	distilled water; $10^{6.3}$ $TCID_{50}$/ml	100%	Elliot & Amend 1978
	25 ppm (1.5 to 40 ppm tested)	30 min	city tap water; PBS; $10^{5.0}$ $TCID_{50}$/ml	100%	Desautels & MacKelvie 1975
	40 ppm (1.5 to 40 ppm tested)	30 min	City water:saltwater; (1:1) $10^{7.5}$ $TCID_{50}$/ml	100%	Desautels & MacKelvie 1975
UV	1500-2000 J per m^2			99.9%	Sako & Sorimachi 1985
	1188±57 J per m^2		freshwater	99.9%	Oye & Rimstad 2001

Method	Dose	Time	Notes	Reduction	Reference
	3367±275 J per m^2		Fish processing plant wastewater	99.9%	Oye & Rimstad 2001
	2460 J per m^2		Filtered seawater	99.9%	Liltved et al 2006
Ozone	1944 (mg s) per liter		Filtered seawater	90%	Liltved et al 2006
	90 mg/(lxh)	0.5 min	Hard lake water	100%	Wedemeyer 2001
	90 mg/(lxh)	10 min	Soft lake water	100%	Wedemeyer 2001
UV-ozone	323-1616 J per m^2 + ozone	3-15 l/min		54-13%	Sako & Sorimachi 1985

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